Radioimmunoassay for the Envelope Glycoprotein of Subgroup E Avian Leukosis–Sarcoma Viruses[1]

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The envelope glycoprotein of subgroup E avian leukosis viruses (gpE) was purified from Rous-associated virus type 0 (RAV-0) and used in double-antibody competition radioimmunoassays (RIA) and radioimmune precipitations (RIP). When embryo extracts from various inbred lines of chickens were tested, the results of assays for chick helper factor (chf) and RAV-0 were in complete phenotypic agreement with those of RIA for gpE. The expression of genes at the *gs* and *gp* loci varied among inbred lines. Normal line $15I_5$ embryos contained gpE but not group-specific (gs) antigens, whereas extracts from virus-free lines 15_1 and 6_1 contained both classes of antigen. Line 15_B embryo extracts were negative for both gpE and gs antigen. Soluble gpE was found in sera of all chf-positive chickens from RAV-0-free lines. Sera from adult line 15_B birds were categorized as: (1) positive for RAV-0 but negative for antibody; (2) negative for RAV-0 but positive for gpE and antibody to gpE; and (3) negative for RAV-0, antibody to gpE, and gpE. Evidence of antibody production was completely correlated in RIP of iodinated gpE and serum neutralizations of subgroup E virus. Results of RIP and gpE RIA indicated that half of the sera from nonviremic line 15_B chickens contained antibodies to gpE as well as gpE.

INTRODUCTION

In the absence of infectious subgroup E avian leukosis–sarcoma (ALS) viruses, some chicken embryo cells contain in their cell membrane the envelope glycoprotein of subgroup E viruses (gpE) (Hanafusa *et al.* 1973). The partial expression of subgroup E virus was first recognized as chick-cell-associated helper factor (chf), which provided the helper function for the envelope-defective Bryan high-titer strain of Rous sarcoma virus (BH-RSV) (Hanafusa *et al.*, 1970a; Weiss and Payne, 1971). Thus, chf acts like a helper virus by determining envelope-antigen specificities such as host range, interference patterns, and neutralizability by antisera.

Embryos from some inbred lines spontaneously release a subgroup E endogenous leukosis virus, Rous-associated virus (RAV-0) (Vogt and Friis, 1971; Crittenden *et al.*, 1974; Robinson and Lamoreux, 1976), or can be induced to release RAV-0 by physical and chemical carcinogens (Weiss *et al.*, 1971), bromodeoxyuridine (Robinson *et al.*, 1976), or dimethyl sulfoxide (Ando and Toyoshima, 1976).

Two apparently independent dominant genes control chf. One, at the group-specific antigen-glycoprotein (*gs*) locus, controls the coordinate expression of internal structural antigens p27, p19, p12, p10 (but not p15) (Weiss and Payne, 1971; Smith *et al.*, 1976), and gpE. The other, at the *gp* locus, controls only the expression of gpE (Hanafusa *et al.*, 1972; Ando and Toyoshima, 1976).

Detection of chf (i.e., gpE) depends on phenotypic mixing in which cellular gpE supplies the missing envelope for BH-RSV (Hanafusa *et al.*, 1970b; Weiss *et al.*, 1971;

Robinson and Lamoreux, 1976). In this report we describe the direct detection of chf in cell extracts and sera from inbred chickens by competition radioimmunoassays (RIA) with anti-RAV-0 serum and iodinated RAV-0 envelope glycoprotein. We also report the simultaneous occurrence of antibodies to subgroup E virus and gpE antigen in RAV-0-free sera from inbred line 15_B chickens.

MATERIALS AND METHODS

Viruses and antisera. The RAV-1 and RAV-2 were obtained from P. K. Vogt. The Prague strain of RSV-C was obtained from R. E. Smith and the Carr–Zilber strain of RSV-D was obtained from R. Duff. The RAV-0 was isolated from a stock of RAV-1 grown in chick embryo fibroblasts (CEF) positive for chf and group-specific (gs) antigens (Rosenthal *et al.*, 1971) and was obtained from H. Hanafusa. The RSV(RAV-0) was obtained from H. Hanafusa and was propagated on line 100_B C/A (resistant to subgroup A virus) cells. Other viruses were propagated on secondary cultures of gs antigen-negative SPA-FAS cells (Spafas, Inc., Norwich, Conn.) in roller bottles.

Chicken antisera to leukosis viruses of subgroups A (No. 682) and E (No. 879) were prepared in line 15_B chickens by natural infection with RAV-1 and by inoculation with RAV-0, respectively. Rabbit anti-chicken immunoglobulin G (IgG) was prepared by subcutaneous injection of an emulsion of 6.4 mg of IgG with an equal volume of Freund's complete adjuvant (Difco, Detroit, Mich.). Three booster injections were given before serum was collected, 2 months after the first injection. Goat anti-rabbit IgG was generously provided by R. Wilsnack and J. Gruber through the Office of Resources and Logistics, National Cancer Institute (Bethesda, Md.).

Inbred chicken lines. This laboratory has maintained lines and sublines for about 38 generations. Some lines have been well characterized for susceptibility to infection and for tumor development after exposure to ALS viruses. Parent stocks have inbreeding coefficients greater than 0.98 and are free of infection with exogenous leukosis virus. Although chickens of line 6 are positive for chf and gs antigens p27, p19, and p12, they are negative for gs antigen p15 and their cells do not produce RAV-0 (Smith *et al.*, 1976). Cells from chickens of line 7_2 produce low titers of RAV-0, but the virus is rarely detected in sera because this line is phenotypically resistant to viruses of subgroups B and E (Crittenden *et al.*, 1973). Chickens of lines $15I_5$ and 15_1 are uniformly positive for chf and resistant to infection by subgroup E viruses, but 20–33% of the embryos spontaneously produce RAV-0 (Crittenden *et al.*, unpublished data). In contrast, chickens of line 15_B are negative for chf and gs antigens, but some embryos and mature birds also spontaneously produce RAV-0 (Crittenden *et al.*, 1977).

Propagation and purification of RAV-0. Line 100 cells that are positive for blood group antigen R1 and spontaneously produce high titers of endogenous RAV-0 (Crittenden *et al.*, 1974) were grown in roller bottles (1330 cm^2) containing 50 ml of an equal mixture of 199 and F-10 media supplemented with 5% tryptose phosphate broth, 4% calf serum, 5% bovine amniotic fluid, and 1% dimethyl sulfoxide. Mycostatin and fungizone were also present at concentrations of 15 and 10 units/ml, respectively. After cell monlayers became confluent, the concentration of calf serum in the medium was reduced to 1%. Culture fluids were collected daily and clarified by centrifugation at 10,000 rpm for 10 min in a Sorvall SS-3 centrifuge. The RAV-0 was pelleted from the supernatant fluids by centrifugation at 20,000 rpm for 75 min with a Beckman Model 21A rotor in a Beckman Model L-3-50 ultracentrifuge. Virus was resuspended in 0.01 vol of TE (0.01 M Tris, 0.001 M ethylenediaminetetraacetic acid [EDTA]), pH 8.2. Suspensions were stored at $-70°$ until enough virus was available for further purification.

Virus glycoproteins were labeled during cultivation of cells in 40 ml of the above-described medium containing 500 μCi of [^{3}H]glucosamine (New England Nuclear,

Boston, Mass.) with 0.02 M fructose instead of glucose (Halpern *et al.*, 1976). A 50-ml pool of 100-fold-concentrated preparations containing labeled RAV-0, prepared as described for unlabeled virus, was further concentrated by centrifugation at 23,000 rpm for 1 hr over a 2-ml "cushion" of 60% (w/v) sucrose. Material at the interface was placed on a continuous 20–60% sucrose gradient and centrifuged for 4 hr at 23,000 rpm in a SW-27 rotor. Glucosamine-labeled fractions with densities of 1.14–1.17 g/ml were pooled and pelleted. About 5 mg of protein (Lowry *et al.*, 1951) was recovered and resuspended in 1.2 ml of TE.

Affinity chromatographic purification of gpE. Lentils, obtained from a local food source, were extracted with water; lectins that precipitated between 33 and 66% saturation with ammonium sulfate were purified by elution with 0.1 M glucose from a 3 × 50-cm column of Sephadex G-100 as described by Howard and Sage (1969). The lectin was lyophilized, and 40 mg was mixed with a suspension of 6 g of cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals) for 2 h at room temperature in 0.1 M NaHCO$_3$ buffer. Unreacted groups on the gel were masked by treatment with M ethanolamine for 2 hr. Three washing cycles were used to remove noncovalently adsorbed protein; each cycle comprised a wash with 0.1 M acetate buffer, pH 4, and a wash with 0.1 M borate buffer, pH 8. Washed, lectin-coupled Sepharose was stored in 0.05% sodium azide at 4°.

The RAV-0 suspension was mixed with 1% desoxycholate, and 0.6 ml of the mixture was incubated at 37° for 2 hr then placed on a 1 × 5-cm column of lectin–Sepharose (Hayman and Crumpton, 1972). Glycoproteins were allowed to adsorb to the immobilized lectin overnight before the column was washed with buffer composed of 0.001 M Tris, 0.01 M NaCl, 0.1% NaN$_3$, and 1% sodium desoxycholate, pH 7.8. After collection of 22 0.6-ml fractions, glucosamine-labeled gpE was eluted in fractions 29–31 with buffer supplemented with 2% α-methyl mannoside.

Iodination and analysis of gpE. About 9 μg of gpE from fraction 30 of the lectin-Sepharose column was iodinated with 0.5 mCi of ^{125}I and chloramine-T as described by Greenwood *et al.* (1963). The purity of mercaptoethanol-reduced, iodinated preparations was evaluated by sodium lauryl sulfate–10% polyacrylamide gel electrophoresis (PAGE) (Fairbanks *et al.*, 1971). Molecular-weight marker proteins used for calibration were: phosphorylase A, 94,000; heavy and light chains of chicken IgG, 67,000 and 22,000, respectively. Sedimentation coefficients for gpE were determined after centrifugation in 5–20% sucrose gradients (Martin and Ames, 1961) with chicken IgG, 6.9 S as a reference standard (Dreesman and Benedict, 1965).

Preparation of anti-RAV-0 serum. Two milligrams of sucrose gradient-purified RAV-0 was solubilized with 0.5 ml of 8 M guanidine hydrochloride and mixed with 1.5 ml of 0.01 M Tris, pH 7.4, and 2 ml of Freund's complete adjuvant. The emulsion was injected subcutaneously into two New Zealand rabbits, at five to seven sites along the back. One rabbit was given three additional series of subcutaneous injections and one intravenous injection during a 4-month period. Serum from the fifth bleeding, obtained 15 weeks after the first injections of this rabbit (designated R205) was selected for use because it had the highest titer of precipitating and virus-neutralizing antibodies.

Titration of antiserum. To 0.1 ml of various dilutions of R205 antiserum were added: 0.1 ml of iodinated gpE probe (about 20,000 cpm); 0.2 ml of pH 7.9 buffer (B-1) composed of 0.01 M Tris, 0.01 M NaCl, 0.005 M EDTA, 0.2% bovine serum albumin, a 1:400 dilution of normal rabbit serum, and 0.2% Triton X-100 (Rohm and Haas, Philadelphia, Pa.); and 0.1 ml of a buffer (B-2) composed of 0.01 M Tris and 0.01 M NaCl. Mixtures were incubated for 3 hr at 37° and then kept overnight at 4°. The next morning, 25 μl of goat anti-rabbit IgG was added; the mixtures were incubated for 1 hr at 37°, kept 3–4 hr at 4°, and centrifuged at 2500 rpm for 15 min. The supernatants were removed by aspiration, and radioactivities from immunoprecipitates were counted in a Beckman

gamma spectrometer. In the absence of competing antigen, about half of the radioactivity put in via the iodinated gpE was precipitated when the R205 antiserum was diluted 800-fold. Serum neutralization tests were conducted as described by Ishizaki and Vogt (1966) with RSV(RAV-0) and line 15$_B$ cells.

Radioimmunoassay of gpE. To 0.1 ml of test material (either chicken serum or twofold dilutions of cell extracts that initially contained about 5 mg of protein/ml) was added 0.1 ml of a 1:800 dilution of R205 antiserum and 0.2 ml of B-1 buffer. After 1 hr at 37°, 0.1 ml of iodinated gpE (about 20,000 cpm) was added. The mixtures were incubated at 37° for 3-4 hr and then kept overnight at 4°. The next morning, 25 µl of goat anti-rabbit IgG was added; the mixtures were incubated for 1 hr at 37°, kept 3-4 hr at 4°, and centrifuged at 2500 rpm for 15 min. The supernatants were removed by aspiration, and radioactivities in pellets were counted in a Beckman gamma spectrometer. In the absence of competing antigen, about half of the radioactivity put in via the iodinated pgE probe was precipitated.

Radioimmune precipitations of gpE. To 0.1 ml of chicken serum, diluted 1:25 to 1:50 in buffer B-2, was added 0.1 ml of iodinated gpE (about 20,000 cpm) and 0.2 ml of B-1 buffer (without normal rabbit serum). After incubation at 37° for 3 hr, the mixtures were kept overnight at 4°. The next morning, 25 µl of rabbit anti-chicken IgG was added, and reaction mixtures were treated as described for antiserum titrations.

Phenotypic classifications. Group-specific antigens were determined by complement-fixation tests (Sarma *et al.*, 1964; Smith, 1977) and by RIA with avian myeloblastosis virus (AMV) polypeptides of MW 27,000 (p27), 19,000 (p19), and 15,000 (p15) (Smith *et al.*, 1976).

For cell-culture assays for RAV-0, cell-free material from either embryo extracts or sera was added to clone 23-16 Japanese quail cells that were transformed by defective BH-RSV (Murphy, 1976), mixed with turkey cells, and seeded in medium containing DEAE-dextran at 2 µg/ml. After 9

days, cell-free culture fluids were assayed on line 15$_B$ cells for RSV(RAV-0). For assays for chf cells were added to B-propiolactone-inactivated Sendai virus and clone 23-16 quail cells. Three days later, cell-free fluids were assayed on 15$_B$ cells for chf. A detailed report on both procedures is in preparation (Randall, Gulvas, and Crittenden).

RESULTS

Purification and analysis of gpE. Essentially all of the nonglycosylated proteins were eluted from a lectin–Sepharose 4B column with the wash buffer in fractions 1-10, but most of the [^{3}H]glucosamine-labeled material was eluted in fractions 29-31 soon after the addition of α-methyl mannoside (Fig. 1). From 3.6 mg of RAV-0 put on the column, about 200 µg of gpE was recovered in these fractions. Inasmuch as AMV gpEs constitute 4-7% of the total virion protein (Bolognesi *et al.*, 1972), almost all of the gpEs were recovered. The results of electrophoretic analysis of iodinated, mercaptoethanol-reduced samples from fraction 30 are shown in Fig. 2. The material in slice 3 is aggregated or incompletely reduced envelope. The major component, with apparent MW 82,000
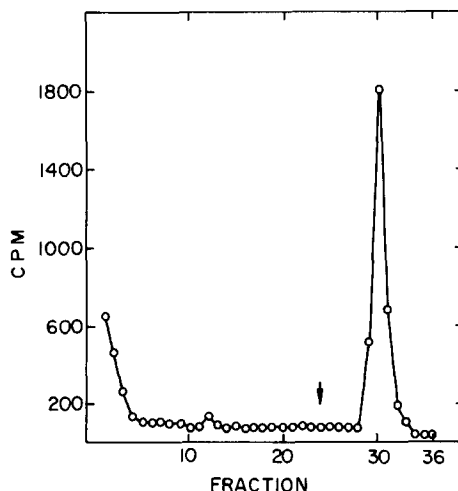

FIG. 1. Purification of glucosamine-labeled glycoprotein from 1% desoxycholate-solubilized RAV-0 by lentil-lectin affinity chromatography. Arrow denotes change to buffer supplemented with 2-methyl mannoside.
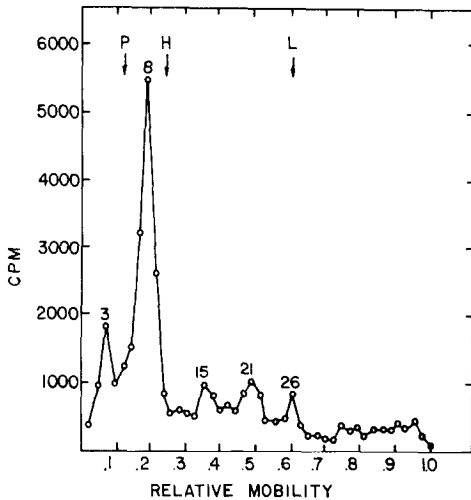
FIG. 2. Gel electrophoresis of iodinated gpE purified by lectin-Sepharose chromatography. Mobilities of MW reference proteins: phosphorylase A, P; heavy and light chains of chicken IgG, H and L, respectively. Numbers above peaks refer to slices.

(slices 7–9), and the material with apparent MW 31,000 (slice 21) are disrupted gpE subunits. About 70% of the total radioactivity placed on the gel was found in these two peaks. Minor components with MW 45,000 and 22,000 (slices 15 and 26) are probably desorbed lentil lectin, because two electrophoretically distinguishable lentil lectins have similar MWs (Howard et al., 1971).

The sedimentation coefficient for iodinated, intact gpE was calculated to be 9.2 S (average of three determinations). This coefficient agrees with that reported for intact viral gpE (Leamnson and Halpern, 1976), except that materials with S values of 12 and 5 were not found in our study. The apparent absence of the latter two gpEs may reflect a different state of aggregation, perhaps due to the different detergents used in lysis of virus. Our preparation was solubilized in 1% sodium desoxycholate, whereas Tween 20 and sodium lauryl sulfate were used by Leamnson and Halpern. If gpE exists as a dimer with two disulfide-bonded chains of apparent MW 122,000 (one heavy chain, MW 85,000; and one light chain, MW 37,000) the combined MW would be 244,000 (Leamnson and Halpern, 1976). From the average sedimentation coefficient of 9.2 S the ap-

proximate MW for gpE was calculated to be 264,000.

The possibility that ALS virus gs antigens contaminated the iodinated gpE probe was ruled out by competition experiments with purified AMV gs antigens. As shown in Table 1, 95% competition was observed with 20 μg of RAV-0 protein, whereas comparable amounts of gs antigens were essentially unreactive. The cross-reactivity of RSV-C and RSV-D may represent sharing of group-specific determinants in envelope antigens.

Titer and specificities of anti-RAV-0 serum. In double-antibody immunoprecipitations, antiserum R205 precipitated about 60% of the total radioactivity put in via the gpE probe (Fig. 3). This corresponded to precipitation of 85% of the gpE probe, inasmuch as PAGE indicated that 70% of the radioactivity was attributable to gpE. Subgroup specificity was also shown by antiserum R205: A 1:10 dilution completely neutralized RSV-0 but not RSVs in subgroups A, B, and C. However, a 1:10 dilution neutralized 94% of RSV-D-induced foci. This cross-neutralization may reflect sharing of group-specific determinants (Halpern et al., 1975).

The specificity of gpE radioimmunoassays with regard to virus subgroups A and B was confirmed by competition tests

TABLE 1

ROUS-ASSOCIATED VIRUS (RAV-0) ENVELOPE GLYCOPROTEIN COMPETITION RADIOIMMUNOASSAYS WITH SOLUBILIZED VIRUSES AND PURIFIED GROUP-SPECIFIC ANTIGENS

Virus or antigen	Subgroup	Protein tested (μg)	Competition (%)
RAV-0	E	20	95
RAV-1	A	25	14
RAV-2	B	24	20
Prague RSV	C	53	40
Carr–Zilber RSV	D	31	48
AMV-p27 [a]	—	20	2
AMV-p19	—	15	7
AMV-p15	—	10	<1
AMV-p12	—	15	<1
AMV-p10	—	5	<1

[a] Chromatographically purified antigens were prepared from the standard strain of avian myeloblastosis virus.
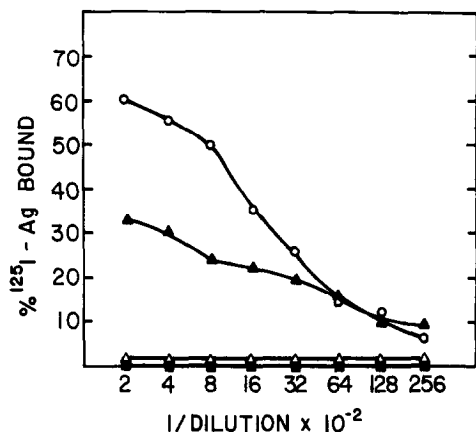
FIG. 3. Double-antibody immunoprecipitations of gpE. Antisera: rabbit (R205), ○; chicken anti-RAV-0, ▲; normal chicken, ■; and chicken anti-RAV-1, △.

representative extracts from inbred lines are illustrated in Fig. 5.

In a survey of embryos from four inbred lines, chf was measured by cell-culture assays and the content of gpE in visceral extracts from the same embryos was determined by RIA. In both assays all RAV-0-free embryos from lines $15I_5$, $15I_4$, and 15_1 had high levels of chf and gpE, whereas embryos from line 15_B were essentially negative for virus antigens (Table 3).

Natural occurrence of soluble gpE in sera. The detection of gpE in RAV-0-negative cell extracts prompted us to examine sera. Three sera from the $15_B \times 7_2$ cross

with concentrated preparations of RAV-0, RAV-1, and RAV-2. Figure 4 shows that 0.1 mg of RAV-0 protein was fully competitive with iodinated gpE, whereas comparable amounts of RAV-1 and RAV-2 protein were essentially noncompetitive. The dose–response curve indicated that the smallest amount of gpE that could be detected, based on 80% binding of the iodinated probe, was about 10 ng. The dose–response curves also indicated that RAV-0 and RAV-60 (not shown) gpEs constitute 6–9% of the total virion protein; these percentages are similar to estimates for other ALS viruses (Bolognesi *et al.*, 1972).

Comparison of cell-culture tests and RIA for RAV-0 and gpE status. For comparison of detection methods, 10- to 11-day-old embryos were tested for endogenous RAV-0 and chf by appropriate cell-culture procedures while extracts from the same embryos were also tested by RIA for gpE, p27, and p15. Although all four embryos from line $15I_5$ tested were negative for RAV-0 and gs antigen, they were positive for chf in both cell-culture tests and RIA for gpE (Table 2). Further, lines 15_1 and 6_1 were positive for gpE and p27, but were negative for p15. Noncoordinate expression of p15 in line 6 has been reported (Smith *et al.*, 1976). Quantitative differences in competition with gpE by
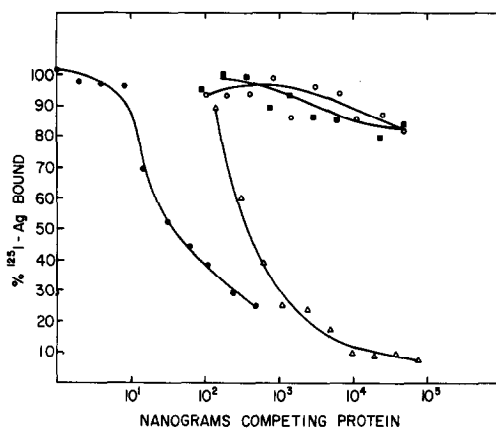

FIG. 4. Competition radioimmunoassays. Displacement of iodinated gpE, ●, by RAV-0, △; RAV-1, ○; and RAV-2, ■.

TABLE 2

CORRELATION OF RADIOIMMUNOASSAY (RIA) RESULTS WITH CELL-CULTURE DETERMINATION OF PHENOTYPES OF CHICKEN EMBRYOS FROM INBRED LINES

Line	Pheno-type[a]	Antigen		
		Envelope	Group specific	
		gpE	p27	p15
$15I_5$	V−, chf+	4/4[b]	0/4	0/4
15_1	V−, chf+	4/4	4/4	0/4
6_1	V−, chf+	4/4	4/4	0/4

[a] Determined as described in Materials and Methods.

[b] With limiting amounts of antibody, less than 80% of maximum bound radioactivity of iodinated envelope glycoprotein (gpE) was precipitated in antigen-positive extracts.
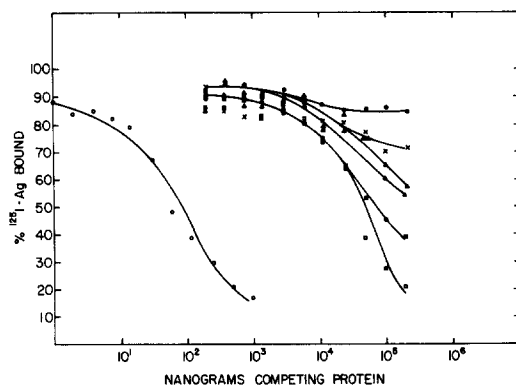
FIG. 5. Competition radioimmunoassays. Displacement of iodinated gpE by gpE, O; by embryo extracts from lines 6_3, □; $15I_5$, ■; 7_2, △; 15_1, ×; 15_B, ●; and a $15I_5 \times 7_2$ cross, ▲.

TABLE 3

CHICK HELPER FACTOR IN VIABLE CELLS AND ROUS-ASSOCIATED VIRUS (RAV-0) ENVELOPE GLYCOPROTEIN (gpE) IN VISCERAL EXTRACTS OBTAINED FROM THE SAME EMBRYO

Line	Number of embryos	chf[a]	gpE[b]
$15I_5$	5	5.4×10^4	277 (165–359)
$15I_4$	5	4.4×10^4	264 (136–340)
15_1	3	6.3×10^4	620 (603–637)
15_B	5	≤ 2	<19

[a] Average number of foci produced per milliliter of supernatant fluids collected 3 days after test cells were fixed with Sendai virus and Rous sarcoma virus (−) quail cells transformed by defective Bryan high-titer.

[b] Average amounts of gpE in nanograms per milligram of protein extracted from viscera. The range is indicated in parentheses.

with high titers of RAV-0 contained gpE as expected, but all sera from RAV-0-negative embryos from lines $15I_5$, 15_1, and 6_3 also contained envelope antigen. Competition for gs antigen probes by sera from the $15_B \times 7_2$ embryos reflects the amplification of endogenous virus production in progeny of this cross (Crittenden et al., 1977) (Table 4). The absence of virus-neutralizing and gpE-precipitating antibodies in sera from embryos from lines $15I_5$, 15_1, and 6_3 and the cross suggests that adult nonviremic chickens are immunologically tolerant to gpE. The extent to which various sera competed with gpE is shown in

Fig. 6. Low titers of RAV-0 are produced by cells of embryos from line 7_2 but RAV-0 is rarely detected in their sera.

Occurrence of gpE and antibodies to gpE in line 15_B sera. Line 15_B embryos and primary cultures are uniformly negative for gs antigen and chf, but a small percentage of embryos produce RAV-0 (Robinson and Lamoreux, 1976; Crittenden et al., 1977; and Crittenden, unpublished). Some line 15_B cultures spontaneously begin to produce RAV-0 after passage, freezing and thawing, or both, and all line 15_B cultures tested produced a noninfectious particle containing reverse transcriptase

TABLE 4

ROUS-ASSOCIATED VIRUS (RAV-0), NEUTRALIZING AND PRECIPITATING ANTIBODIES TO ENVELOPE GLYCOPROTEIN (gpE), AND GROUP-SPECIFIC ANTIGEN (gs) IN CHICKEN SERA

Line or cross	Antibodies				
	RAV-0[a]	SN[b]	RIP[c]	gpE[d]	gs[e]
$15I_5$	1/5	0/5	0/5	5/5	0/5
15_1	3/5	0/5	0/5	5/5	0/5
6_3	0/4	0/4	0/4	4/4	0/4
$15_B \times 7_2$	3/3	0/3	0/3	3/3	2/2
7_2	0/4	0/4	0/4	0/4	0/4

[a] Determined by RAV-0 assays after addition of serum cell cultures susceptible to subgroup E virus.

[b] Serum neutralization of RAV-0.

[c] Radioimmune precipitation of iodinated gpE.

[d] Determined by gpE competition radioimmunoassay (RIA).

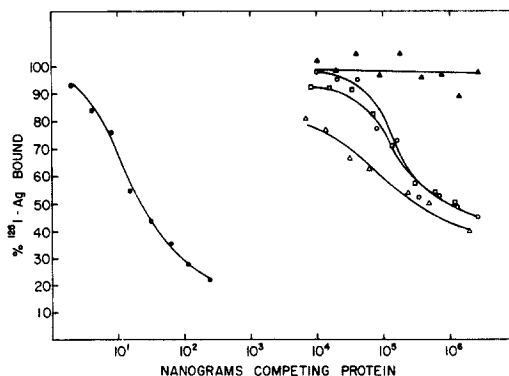[e] Determined by p27 and/or p19 competition RIA.

FIG. 6. Competition radioimmunoassay. Displacement of iodinated gpE, ●; by sera from line 7_2, ▲; line 6_3, O; line 15_B, △; and a $15_B \times 7_2$ cross, □.

after treatment with bromodeoxyuridine (Robinson *et al.*, 1976; Crittenden *et al.*, 1977). We also observed in this study that many adult line 15$_B$ chickens are viremic with RAV-0 or have neutralizing antibodies to subgroup E. Twenty-seven sera from previously characterized chickens were further assayed for gpE, antibody to gpE, and p27 by RIA (Table 5). The sera were categorized into three classes: (1) sera with no evidence of virus either in cell-culture assays or in RIA; (2) sera negative for RAV-0 but positive for neutralizing and precipitating antibodies and gpE; and (3) sera viremic with RAV-0 but negative for neutralizing and precipitating antibodies. The last was the only class in which p27 was detected, in agreement with the presence of RAV-0 in these sera. Neutralization tests for antibodies to subgroup E virus and immune precipitations of gpE were also in complete agreement. The RIA data also showed that gpE was present in antibody-positive, RAV-0-negative (class 2) sera, suggesting the presence of antigen–antibody complexes.

DISCUSSION

The glucosamine-labeled protein isolated by lentil-lectin affinity chromatography had a sedimentation coefficient of approximately 9.2 S and thus represents intact viral envelope glycoprotein. After iodination, electrophoresis of reduced protein revealed a major component of apparent MW of 82,000. Because sedimentation analyses of undenatured glycoprotein in-

TABLE 5

ROUS-ASSOCIATED VIRUS (RAV-0), NEUTRALIZING AND PRECIPITATING ANTIBODIES TO ENVELOPE GLYCOPROTEIN (gpE), gpE, AND GROUP-SPECIFIC ANTIGEN p27 IN LINE 15$_B$ SERA[a]

Serum class	RAV-0	Antibodies		gpE	p27
		SN	Ab RIP		
(1) Ab(−)	0/11	0/11	0/11	0/11	0/9
(2) Ab(+)	0/11	11/11	11/11[b]	11/11	0/4
(3) Ab(−)	5/5	0/5	0/5	1/5	3/5

[a] For methods, see footnotes to Tables 2 and 4 and Materials and Methods.

[b] At 1:50 dilutions, 31–46% of iodinated gpE was precipitated with [Ab−]positive sera.

dicated a MW considerably greater than 82,000, we designate this component of RAV-0, the prototype subgroup E virus, gpE.

Heterologous immune precipitations of sarcoma viruses belonging to subgroups A and C by antiserum against avian myeloblastosis-associated virus B (Rohrschneider *et al.*, 1975) and of gpE in chf-positive cells by antiserum against a subgroup C strain of RSV indicate that different ALS viruses share group-specific envelope-antigen determinants (Halpern *et al.*, 1975). Subgroup classification is based on host range and interference patterns, but neutralization of only certain viruses within a subgroup by antisera to cloned viruses and the ability of mutant viruses to grow in heterologous avian cells indicate that type-specific envelope-antigen determinants also exist in viruses within the same subgroup (Vogt, 1970; Zarling *et al.*, 1977).

The gpE RIA used in this study was essentially type specific for subgroup E viruses; competition by RAV-0 and RAV-60 was efficient at high dilutions, whereas competition by low dilutions of RAV-1 and RAV-2 was negligible. The relatively high competition for gpE by the Carr–Zilber strain of RSV-D is consistent with the strong interference between subgroup D and E viruses reported by Hanafusa *et al.* (1970b). Antiserum R205 also contained antibodies to gs antigens, but the absence of competitition by solubilized preparations of RAV-1 and RAV-2 confirmed that the gpE probe was free of gs antigens. Contamination of gpE with reverse transcriptase is also unlikely inasmuch as antiserum to AMV reverse transcriptase did not precipitate iodinated gpE (data not shown). The agreement between the biological assays for chf and RAV-0 and the RIA for gpE also indicated that similar if not identical envelope antigens were measured.

The extent of virogene expression in embryos differed markedly among lines. The RAV-0 free embryos from line 15$_1$ resembled those from lines 6$_1$ and 6$_3$ in the uniform expression of gpE and p27 but not p15. Line 6 embryos, however, are neither inducible nor spontaneous pro-

ducers of RAV-0, whereas about 33% of line 15₁ embryos spontaneously produce RAV-0. We do not know whether information for p15 is deleted from the *gs* gene in line 6 or whether failure to produce RAV-0 is due to differences in regulatory genes. In this context, the provirus may not be under stringent control in line 15 cells because about 20% of the line 15I₅ gpE-positive embryos also produce RAV-0.

Finding gpE in sera from chf-positive lines was not unexpected, because envelope glycoprotein gp 69/71 of murine leukemia virus has been reported in sera of inbred mice (Yoshiki *et al.*, 1974; Lerner *et al.*, 1976; Strand and August, 1976). Of the 22 nonviremic line 15ᴮ sera tested, half contained gpE as determined by competition RIA and gpE antibodies as determined by serum neutralization and RIP. Whether gpE exists totally as an antigen-antibody complex or excess antigen remains nonbound remains to be determined. It is possible that R205 antiserum may recognize gpE determinants that are not occupied by chicken antibodies and "sandwich" complexes competed with the probe.

Inasmuch as some line 15ᴮ cells and chickens also spontaneously produce RAV-0 (Crittenden *et al.*, 1977), the viremic, antibody-negative chickens may have produced RAV-0 as embryos and thus have remained immunologically tolerant. Others may have spontaneously produced RAV-0 or were infected by contact, experiencing a transient viremia with subsequent antibody production. Alternatively, the gene at the *gp* locus may have been activated after hatching to induce antibody production in these birds; autogenous immunity to envelope antigen of endogenous murine RNA tumor virus has been reported (Hanna *et al.*, 1972). Experiments are planned to test these alternatives.

Supported by assays for RAV-0 our results show that the RIA for gpE can be used instead of the cell-culture assay for chf in the phenotypic classification of cells and lines. When embryos express p15, one may assume that RAV-0 or another infectious ALS virus is replicating in the cells or present in the serum. Usually, however, too little RAV-0 is present for detection of p15 by RIA, even though the more sensitive cell-culture RAV-0 assay can detect infectious subgroup E virus.

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